The Resistivity of Microorganisms to Thermal Inactivation by Dry Heat

May 5, 1967

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Contract No: NSG--691/22-009-048

Submitted to:

Office of Grants and Research Contracts
Office of Space Science and Applications
Attention: Code SC
National Aeronautics and Space Administration
Washington, D.C. 20546

It has been established from previous experiments by this investigator and others that water is one of the most important moderator in thermal inactivation by dry heat. Two approaches are being employed to ascertain the extent to which water mediates in the kinetics of inactivation by equilibration. One approach is to condition the spore crop at various water activities prior to the exposure to standard conditions in the experimental heating chamber. The second approach is to expose preconditioned spores to heated atmospheres containing various moisture levels at specific air velocities. In this manner it is anticipated that an analysis of the kinetics of inactivation will impart an insight into mechanisms involving the role of water in inactivation. One factor which should strongly influence experimental results is the nature of the support material and its ability to alter the influence of water in thermal inactivation. Four support materials of different porosity and surface characteristics have been investigated to date. These include membrane filters, glass fiber filters, glass cover slips and stainless steel. Additional factors which might be of importance in investigating the influence of water in thermal inactivation are the purity of the spore preparation and the physiological state of the spore. To date the presence of the normal quantity of cellular debris

in the spore crop has not been found to be a major factor in spore resistivity but the physiological state of the spore crop has been found to be of considerable importance. Certain spore crops have been found to possess greater resistivity. Of more importance has been a periodic loss of resistivity during prolonged refrigerated storage in water without any apparent reason.

To date complete inactivation curves for Bacillus subtilis var. niger have been derived for membrane filters and fiber glass filters. Present efforts have been devoted to glass cover slips. We are now in the process of deriving D and Z values for dry heat, under static conditions and the ability of air velocity, moisture and inert gases to alter these values.

A description of the oven and the methods for establishing equilibrium between the spores and the support material has been presented in previous reports. The present spore crop was grown in a 15 liter fermenter with constant aeration and the spores separated from vegetative debris by the method of Sacks + Alderton (1961, J. Bacteriol. 82, 331). Certain portions of the spore crop were freeze-dried in flasks or on cover slips and aliquots were also equilibrated in phosphate buffer (pH 7.0) or in $CaCl_2$ (pH 7.0) for one week, washed and resuspended in glass distilled water.

For the heat inactivation study on stainless steel the method of Subcommittee I on Spacecraft Sterilization was followed with the following exception. The removal of the spores from the stainless steel support was accomplished by exposing the strip to a Branson 125 watt ultrosonicator at a setting of 6 for two minutes. The probe had a 1/8 inch tip and the temperature during sonication was maintained at 0-5°C. Care was taken to always have the inoculated surface facing the probe during sonication.

To remove spores from the glass cover slides either blenderizing for two minutes or crushing followed by vortexing was employed. Recovery was essentially equal for these two methods on control (unheated) samples and on heated samples.

RESULTS

As seen in Figure 1 the resistivity of Bacillus subtilis var. niger on glass cover slips heated at 125° under static conditions was less than spores heated on glass fiber filters. In both cases the spores were equilibrated over silica gel (assumed to be at o% RH). The D value is approximately 33 minutes for glass fiber filters and 15 minutes for glass cover slips.

The influence of water activity on thermal resistivity is illustrated in Figure 2. One of the spore crops in Figure 2 possessed a higher resistivity at 125°C whereas the other had, to a considerable extent, lost its intrinsic resistivity. It is seen that in both cases minimal resistivity occurs in the range of 0-15% RH and maximal resistivity at 80% RH. It should be noted that the RH considered here refers to the RH of the spores prior to exposure to 125°C in the oven and not that RH which exists in the oven during heating. From the values derived from Figure 2 increasing the equilibrium RH to 80% increases the D value for the resistant spore crop to approximately 72 minutes.

That an optimum for survival exists at approximately 80% RH may not be too surprising. As seen below for experiments on the effect of RH on the survival of spores exposed to heat it is conceivable that the observed protection effect of water may be a net result and that under certain conditions water may be detrimental to survival.

Spores on glass cover slips were also equilibrated at either 0% RH or 33% RH and, after heating, reequilibrated at various RH's (Figure 3) the effect of RH is not as dramatic in this instance as for spores treated in Figure 2.

Spores equilibrated at 0% RH showed a decreased survival if re-equilibrated after heating at 33% RH. In contrast to this maximal recovery was noted for spores which had been equilibrated at 33% RH prior to heating and then at 33% RH after heating. These results are not easy to interpret but in any event this data does show that the recovery procedure normally employed in these experiments does not produce any significant artifact. In this case, high moisture levels does not appear to drastically reduce recovery. Additional experiments are needed, though, to more thoroughly verify this point.

In further attempts to alter resistivity to dry heat, the spore suspension was freeze-dried prior to placement on cover slips, after replacement on cover slips and equilibrated for one week in phosphate buffer or in calcium chloride. Freeze-drying had no appreciable effect in resistivity in these experiments if freeze-dried after inoculated onto cover slips prior to heating. If freeze-dried in bulk, rehydrated and then dried on cover slips prior to heating, the resistivity was appreciably lowered. Equilibration in phosphate did not seem to lower resistivity whereas calcium lowered thermal resistivity. In the experiments involving either phosphate

or calcium the spores were washed prior to drying on the support material and then equilibrated over silica gel. Future experiments will involve soaking spores in DPA.

The D value obtained at 125°C on stainless steel strips equilibrated at 0% RH was 15.5 minutes (Figure 4).

It is therefore apparent that in addition to water the support material exerts a real and finite influence upon the survival of spores of Bacillus subtilis var. niger. This is shown in Table 1. Those materials which are more porous and which present more surface area and surface forces will prolong survival.

Examination of the various curves presented in this report indicates that one characteristic of the heating chamber is to impart a shoulder to the inactivation curve at 125°C. This was true for heating either in static or flowing air. It is difficult to explain this fact unless one wishes to assume either an artifact due to the initial retention of small quantities of water within the chamber (a protective effect) or a finite requirement for mass transfer to the spores with time.

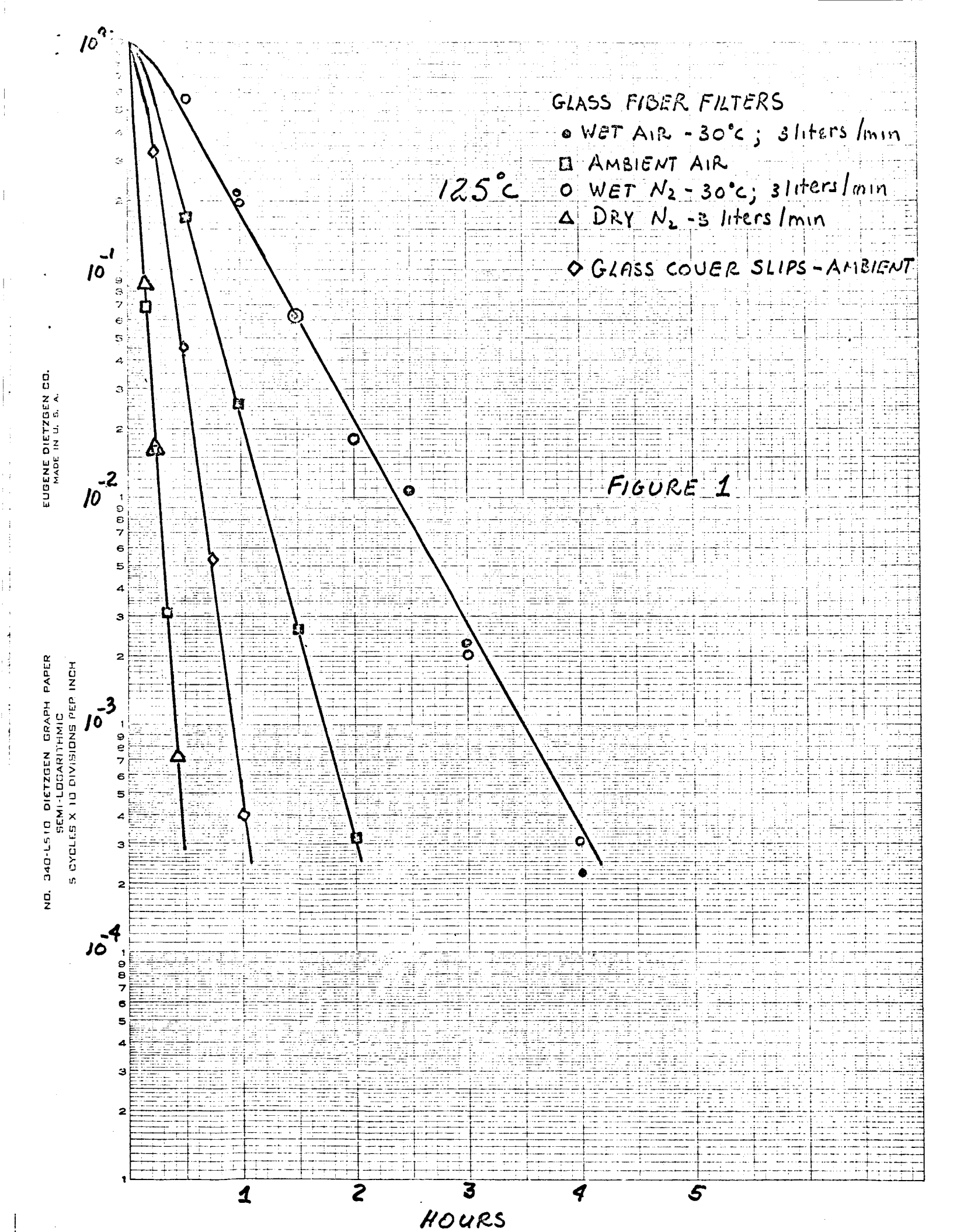
One factor which is not in agreement with certain other investigators is the fact that freeze-drying inoculated spores on cover slips prior to heating did not alter resistivity nor was there a change in the inactivation curve indicating the

establishment of a more resistant spore population. The substantiation of this will require additional experimentation.

The response of the different spore crops in Figure 2 was of interest. The less heat resistant spore crop had a higher response to RH than the more resistant spore crop. This is evident when one compares the survival fraction at 0% RH and 80% RH. It is conceivable that as regards dry heat inactivation water may mediate in stabilizing certain cellular consituents necessary for resistivity which would be more apparent with spores which have lost some of their intrinsic resistivity due to a loss of cellular constituents or to modification of intermolecular spacial relationships.

Table 1. The D values of *Bacillus subtilis* var. *niger* at 125°C on various support materials. The equilibruim relative humidity was established by silica gel (0% RH)

	D Value (minutes)
Glass Fiber Filters	33
Glass Cover Slips	15
Stainless Steel	16


GLASS FIBER FILTERS
• WET AIR - 30°C ; 3 liters/min
□ AMBIENT AIR
125°C
○ WET N_2 - 30°C; 3 liters/min
△ DRY N_2 - 3 liters/min
◇ GLASS COUER SLIPS - AMBIENT
FIGURE 1
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
1
2
3
4
5
HOURS
EUGENE DIETZGEN CO.
MADE IN U. S. A.
NO. 340-L510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH

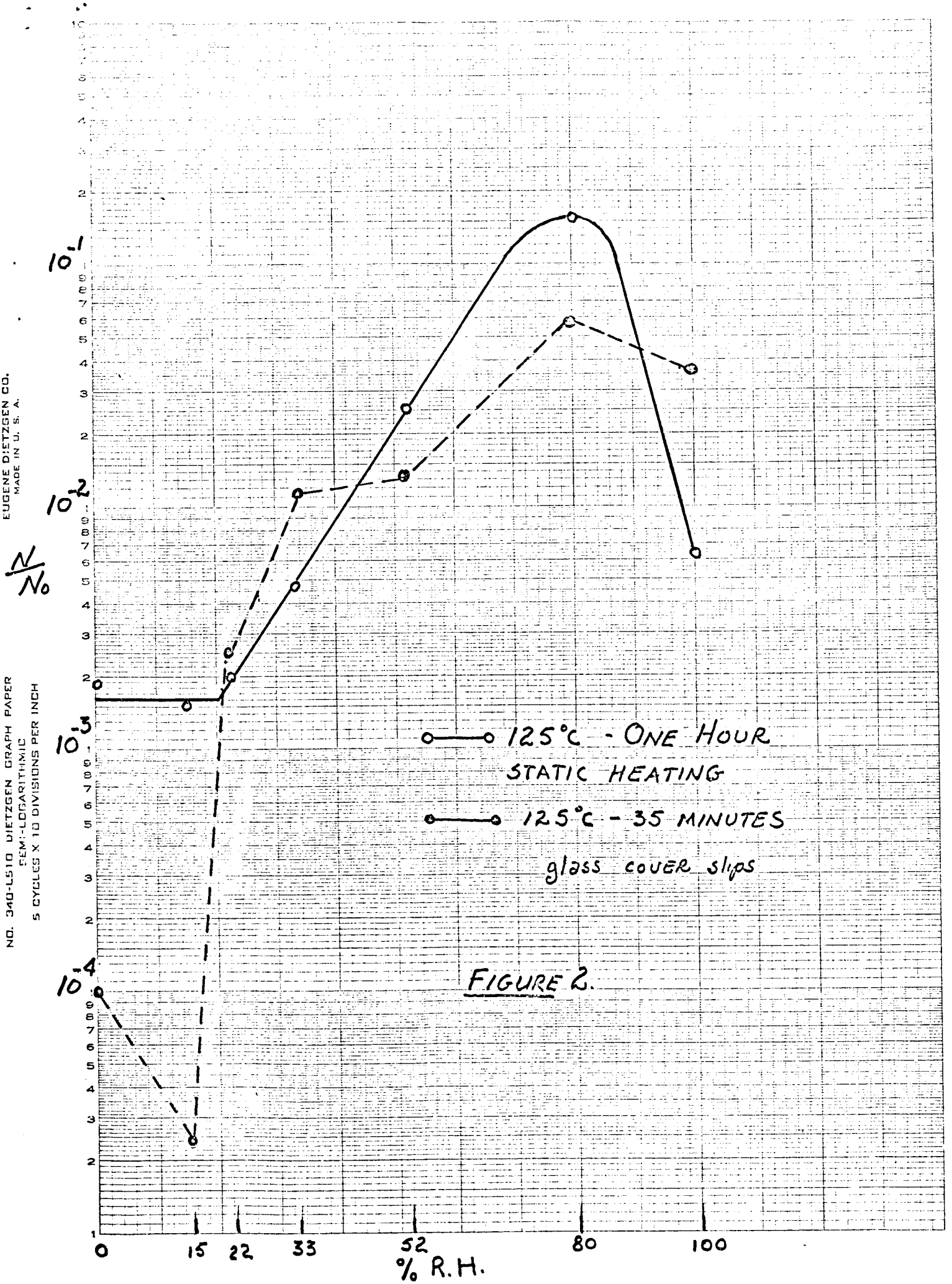

N/No
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
15
22
33
52
80
100
% R.H.
125°C - ONE HOUR
STATIC HEATING
125°C - 35 MINUTES
glass cover slips
EUGENE DIETZGEN CO.
MADE IN U. S. A.
NO. 340-L510 DIETZGEN GRAPH PAPER
SEMI-LOGARITHMIC
5 CYCLES X 10 DIVISIONS PER INCH

FIGURE 2.

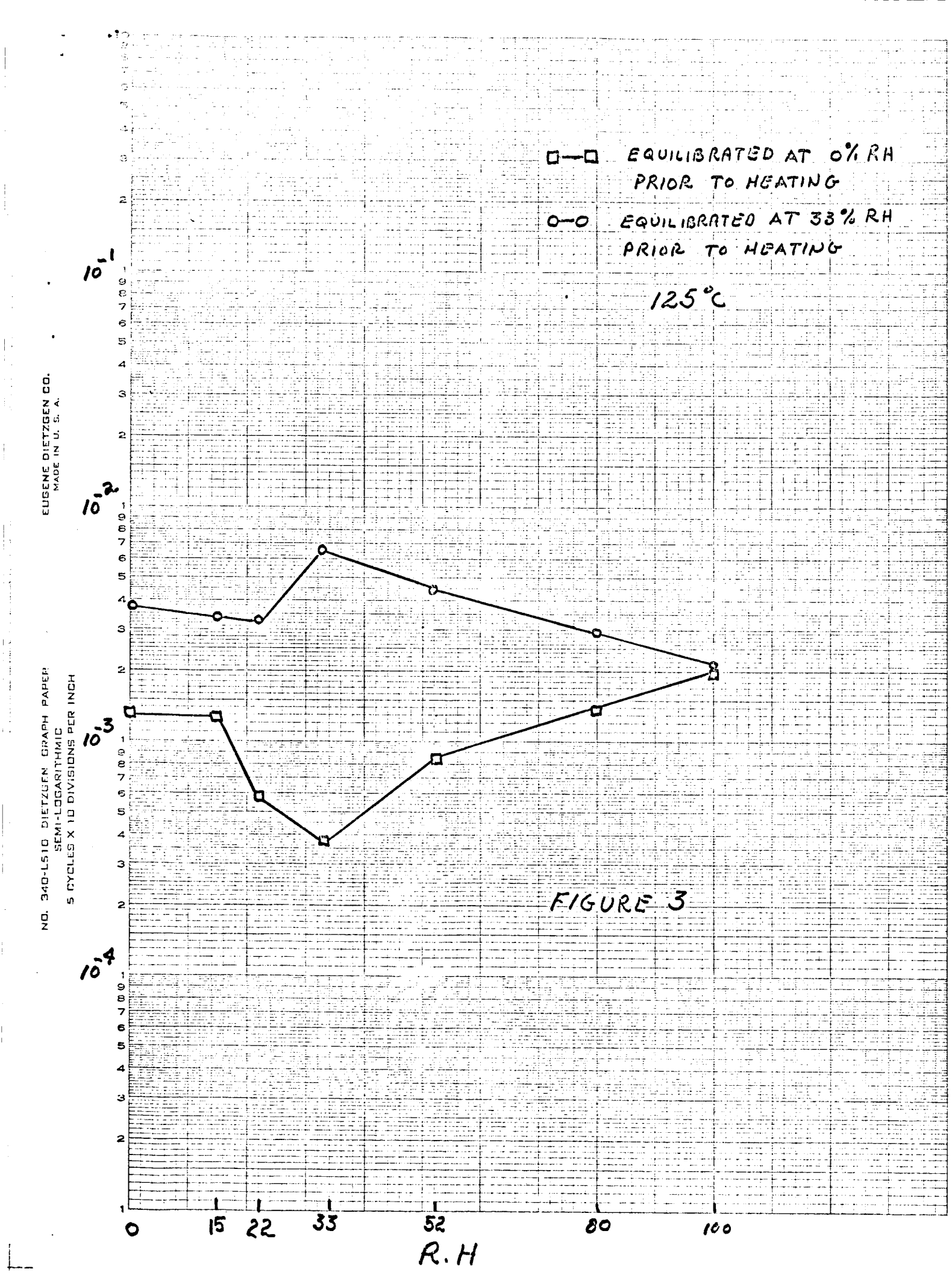
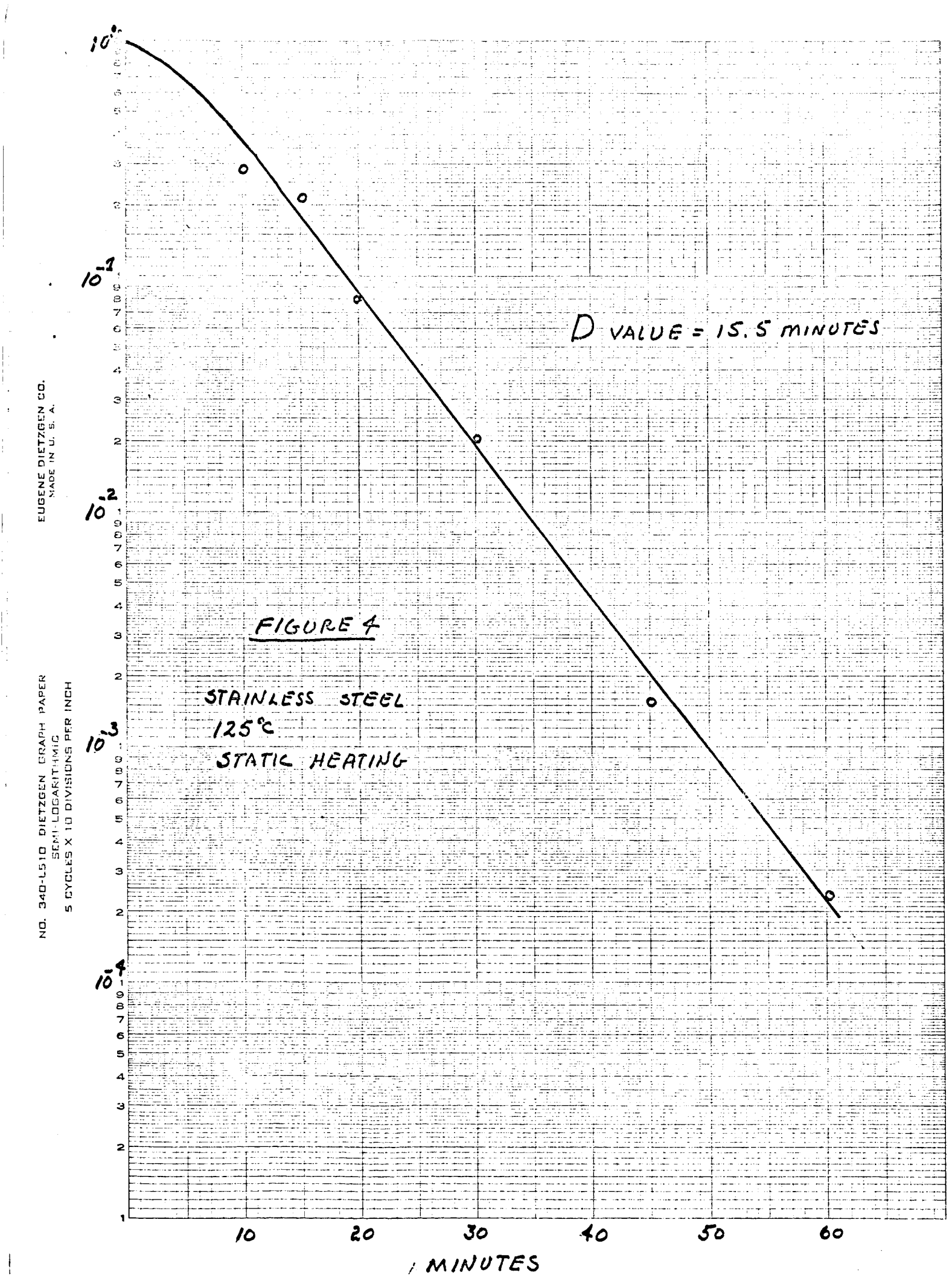

□—□ EQUILIBRATED AT 0% RH PRIOR TO HEATING
o—o EQUILIBRATED AT 33% RH PRIOR TO HEATING
125°C
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
15
22
33
52
80
100
R.H
EUGENE DIETZGEN CO. MADE IN U. S. A.
NO. 340-L510 DIETZGEN GRAPH PAPER SEMI-LOGARITHMIC 5 CYCLES X 10 DIVISIONS PER INCH

FIGURE 3


D VALUE = 15.5 MINUTES
FIGURE 4
STAINLESS STEEL
125°C
STATIC HEATING
10
20
30
40
50
60
MINUTES
EUGENE DIETZGEN CO.
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